FINAL REPORT

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In Situ Hydrocarbon Degradation By Indigenous Nearshore Bacterial Populations

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Please be advised that there is an error in the title of the soft landing grant number DE-FG02-01ER63092 in the amount of $74,962 awarded to Dr. Jennifer Cherrier in the Environmental Sciences Institute at Florida A&M University. The title of the grant now erroneously reads "Tracing Carbon and Nitrogen Cycling by Indigenous Marine Bacterial Populations Using Geochemical and Molecular Approaches". This was the title of the BI-OMP proposal that Dr. Cherrier wrote together with Drs. Young, Proctor, and Chanton. This proposal was not funded. Dr. Cherrier was given a soft landing award, however, and was asked to write a separate two page proposal for this soft landing as well as submit a new set of DOE forms (i.e. face page, budget etc...) and budget justification on which she was the sole principal investigator. She did so and the title of this soft landing proposal, as listed on DOE forms DOEF 4650.2 and DOEF4620.1, is "In Situ Hydrocarbon Degradation By Indigenous Nearshore Bacterial Populations". I am not sure if this error was ever corrected although A. Palmisano did send a letter to Joseph N. Zameic, Contract Specialist Acquisition and Assistance Group in the Department of Energy Chicago Operations Office March 16, 2001.

Potential episodic hydrocarbon inputs associated with oil mining and transportation together with chronic introduction of hydrocarbons via urban runoff into the relatively pristine coastal Florida waters poses a significant threat to Florida's fragile marine environment. It is therefore important to understand the extent to which indigenous bacterial populations are able to degrade hydrocarbon compounds and also determine factors that could potentially control and promote the rate at which these compounds are broken down *in situ*. Previous controlled laboratory experiments carried out by our research group demonstrated that separately both photo-oxidation and cometabolism stimulate bacterial hydrocarbon degradation by natural bacterial assemblages collected from a chronically petroleum contaminated site in Bayboro Bay, Florida. Additionally, we also demonstrated that stable carbon and radiocarbon abundances of respired CO_2 could be used to trace *in situ* hydrocarbon degradation by indigenous bacterial populations at this same site. This current proposal had two main objectives: a) to evaluate the cumulative impact of cometabolism and photo-oxidation on hydrocarbon degradation by natural bacterial assemblages collected the same site in Bayboro Bay, Florida and b) to determine if in situ hydrocarbon degradation by

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indigenous bacterial populations this site could be traced using natural radiocarbon and stable carbon abundances of assimilated bacterial carbon. Funds were used for 2 years of full support for one ESI Ph.D. student, April Croxton.

To address our first objective a series of closed system bacterial incubations were carried out using photo-oxidized petroleum and pinfish (i.e. cometabolite). Bacterial production of CO_2 was used as the indicator of hydrocarbon degradation and $\delta^{13}C$ analysis of the resultant CO_2 was used to evaluate the source of the respired CO_2 (i.e. petroleum hydrocarbons or the pinfish cometabolite). Results from these time series experiments demonstrated that short-term exposure of petroleum to UV light enhanced hydrocarbon degradation by 48% over that observed for non-photo-oxidized petroleum. Despite the greater bio-availability of the photo-oxidized over the non-photo-oxidized petroleum, an initial lag in CO_2 production was observed indicating potential photo-toxicity of the photo- by-products. $\delta^{13}C$ analysis and mass balance calculations reveal that co-metabolism with pinfish resulted in increased hydrocarbon degradation for both photo-oxidized and non-photo-oxidized petroleum each by over 100%. These results demonstrate the cumulative effect of photo-oxidation and co-metabolism on petroleum hydrocarbon degradation by natural bacterial populations indigenous to systems chronically impacted by hydrocarbon input.

To address the second objective of this proposal bacterial concentrates were collected from Bayboro Harbor in April 2001 for nucleic acid extraction and subsequent natural radiocarbon abundance analyses. Unfortunately, however, all of these samples were lost due to a faulty compressor in our ¯70 freezer. The freezer was subsequently repaired and samples were again collected from Bayboro Harbor in June 2002 and again December 2002. Several attempts were made to extract the nucleic acid samples-however, the student was not able to successfully extract and an adequate amount of un-contaminated nucleic acid samples for subsequent natural radiocarbon abundance measurements of the bacterial carbon by accelerator mass spectrometry(i.e. require at least 50μg carbon for AMS measurement). Consequently, we were not able to address the second objective of this proposed work.

GRANT RELATED PUBLICATIONS AND PRESENTATIONS

Published Manuscripts and Abstracts

McCallister, S.L., Bauer, J.E., Cherrier, J., and H. Ducklow. (2004). Assessing sources and ages of organic matter supporting river and estuarine production: A multiple isotope ($\Delta^{14}C$, $\delta^{13}C$ and $\delta^{15}N$) approach. Limnology and Oceanography, 49 (5): 1687-1705

Clarke, M.A., Gragg. R., Cherrier, J., and J. Chanton. Determination of the impact of ultraviolet radiation, cometabolism, and bioaugmentation on the degradation of petroleum by marine bacteria. American Society of Limnology and Oceanography Winter Meeting. Salt Lake City, UT. Feb. 2003

Hepburn, C., Cherrier, J. and J. Chanton. Tracing In Situ Petroleum Hydrocarbon Utilization Using Natural Carbon Isotope Abundances. American Society of Limnology and Oceanography Winter Meeting. Salt Lake City, UT. Feb. 2003

Manuscripts In Review or In Preparation

Cherrier, J. and C. Hepburn. Tracing *in situ* petroleum hydrocarbon utilization using natural carbon isotope abundances. Environmental Science and Technology. In Prep

Clarke, M.A., Cherrier, J., Chanton, J., Robinson, L., and R. Gragg. Using respiration and

stable istotopes to evaluate the impact of photooxidation and cometabolism on petroleum biodegradation. Environmental Science and Technology, submitted.